Microfungal succession on living leaves of *Populus tremuloides*

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The microfungal succession on the surfaces and interior of attached aspen poplar leaves was followed throughout a growing season at three heights in the tree canopy using a number of isolation and observational techniques. The fungal succession on and within the leaves was in general similar to that reported for other angiosperm leaves. The outer sheathing bud scales were colonized by a limited range of fungi, but the enclosed leaves were free from fungal colonization. The adaxial surfaces of young newly expanded leaves were sparsely colonized by fungi. As the leaves matured they were extensively colonized by the common phylloplane fungi (pink and white yeasts, *Aureobasidium pullulans*, *Cladosporium* spp., *Alternaria alternata*, and *Epicoccum purpurascens*) especially on the adaxial surface. At senescence both leaf surfaces had extensive hyphal growth on them and a greater internal colonization by phylloplane fungi had occurred. Common phylloplane fungi were less frequently isolated from freshly fallen leaves than from the senescent leaves, but a sterile dark species was frequently isolated from within them. The height of the leaves in the canopy was shown to influence the mycoflora of the aspen poplar leaves, with certain species (the yeasts, *Aureobasidium pullulans*, *Cladosporium* spp., and *Botrytis cinerea*) showing changes in their frequency with sampling height.

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La succession microfongique à la surface et à l'intérieur de feuilles attachées de peuplier faux-tremble a été suivie à l'aide de diverses méthodes d'isolement et d'observation, pendant toute la saison de croissance et à trois hauteurs dans la cime de l'arbre. La succession fongique à la surface et à l'intérieur des feuilles est, en général, semblable à celle qu'on a décrite chez d'autres feuilles d'angiospermes. Les écailles engainantes du bourgeon sont colonisées par une gamme limitée de champignons, mais les feuilles à l'intérieur du bourgeon sont dépourvues de toute colonisation fongique. La surface adaxiale des jeunes feuilles récemment déployées sont peu abondamment colonisées par des champignons. A mesure que les feuilles poursuivent leur maturation, elles deviennent fortement colonisées par les champignons phylloplanes communs (levures roses et blanches, *Aureobasidium pullulans*, *Cladosporium* sp., *Alternaria alternata*, *Epicoccum purpurascens*), surtout sur la surface adaxiale. Au moment de la sénescence, les deux surfaces foliaires sont couvertes d'une quantité considérable d'hyphes et il y a une colonisation interne plus importante par les champignons phylloplanes. Les champignons phylloplanes communs ont été isolés moins fréquemment des feuilles frâchement tombées que des feuilles sénéscentes, mais on en a fréquemment isolé une espèce stérile foncée. La hauteur des feuilles dans la cime influence la mycoflore des feuilles de peuplier faux-tremble; la fréquence de certaines espèces (levures, *Aureobasidium pullulans*, *Cladosporium* spp. et *Botrytis cinerea*) change avec la hauteur de l'échantillon dans la cime.

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Introduction

In the past 2.5 decades there has been a considerable accumulation of information on the physiological activity and succession of fungi which bring about the decay of plant remains above the soil. Hudson's (1968) and Sharma and Mukerji's (1973) reviews and Dickinson and Pugh's (1974) book detail much of the fungal succession work carried out on a wide variety of different plant materials. Studies beginning in the late 1950's have shown that an active fungal population exists on the surface and within physiologically active green leaves (Kerling 1958, 1964; Ruinen 1961; Last and Deighton 1965; Dickinson 1965, 1967; Last and Warren 1972). Recent books (Preece and Dickinson 1971; Dickinson and Preece 1976) have reviewed the work concerning the microbial colonization of aerial plant (and in particular leaf) surfaces, and the influence of microorganisms, including phylloplane fungi, on leaf processes and on overall forest ecosystem activities, has been emphasized by several authors (Dickinson 1976; Dickinson and Wallace 1976; Dickinson and O'Donnell 1977; Bernstein and Carroll 1977).

Over the course of the last few years detailed studies on the decomposition of aspen poplar leaves have been published (e.g., Visser and Par-

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kinson 1975; Lousier and Parkinson 1976, 1978); however, little data have been given on the associations of fungi with live and senescent aspen poplar leaves. Therefore, apart from intrinsic interest, the present study was undertaken to provide data on the fungal succession on and within aspen poplar leaves throughout a growing season and to determine the major fungal saprophytes and parasites (with respect to frequency of occurrence) present. In addition, the leaf mycoflora was examined at several heights in the tree canopy to determine the effect of height on the fungal succession. The data obtained were subjected to statistical analysis, an approach which has unfortunately been lacking in many fungal succession studies.

This work was also the initial step in a study aimed at examining the influence of leaf leachate carbohydrates, in their role as substrates, on phylloplane fungal behaviour.

TABLE 1. Outline of sampling strategy

Date	Sample	Sample heights, m
4 May	Buds* (1) outer bud scales (2) enclosed leaves	5
3 June	Young leaves (newly expanded)	5
10–11 August	Mature leaves	5, 8, 12
2 October	Senescent leaves (yellow in appearance)	5, 8, 12
11 October	Freshly fallen leaves†‡	—

*Leben (1971) noted that buds may be washed and the washings cultured for assay, but this method probably detects few organisms on the interior parts. Maceration methods do not permit the distinction between organisms within bud tissues from those on the surface, and the effect of the chemical components of the leaf and their oxidized derivatives on microbial growth and development is not known. For these reasons, buds were dissected apart and the outer bud scales treated separately from the internal parts.

†Freshly fallen leaves were caught in large nylon nets suspended from the trees. This enabled the leaves to be collected before they had made contact with the litter surface, hence they were probably free from contamination by litter microorganisms.

‡The replicates for this sample all came from one subplot, due to net damage caused by large mammals in the other two subplots.

Materials and Methods

Study Area

The sampling area is located in the Kananaskis Valley in the front range of the Rocky Mountains (51°2′ N, 115°2′ W) in Alberta, Canada, at an altitude of 1430 m. A description of the site and vegetation is provided by Visser and Parkinson (1975) and climatic data are provided by Mitchell (1974). A sampling area containing three subplots was marked out in a mature aspen poplar stand. The three subplots were chosen because of their similarity in almost all respects (viz. aspect, tree age, height and density, and associated vegetation) and were treated as statistical replicates for all the tests.

Sampling

Leaves from 12 aspen poplar trees in each of the three subplots were collected at intervals throughout the 1976 growing season. Five stages in the leaf life cycle were examined with leaves being sampled from a standard height on the trees on all occasions. To determine if there was a variation in the leaf mycoflora with height in the tree canopy, leaves were sampled at three heights in the canopy on two occasions during the sampling period. The sampling strategy is detailed in Table 1. After collection, leaves were transported back to the laboratory in new polythene bags and stored (2–7 days) at 5°C before processing.

Fungal Population Analysis

A combination of isolation and direct observation methods was employed to obtain as comprehensive a picture as possible of the composition of and changes in the leaf mycoflora. In addition, techniques were employed to differentiate between colonists of the phylloplane and leaf interior.

The isolation medium employed for all cultural studies was 2% malt agar (Tuite 1969) amended with 50 mg/L aureomycin and 100 mg/L streptomycin to eliminate bacteria (hereafter referred to as MA^+). Preliminary experiments with several media had shown malt agar to isolate the largest spectrum of fungi and also to facilitate rapid fungal growth.

(a) Washed Leaves

Leaf discs were subjected to a washing procedure in an attempt to remove all easily detachable propagules from the leaf surface. One-hundred 5-mm diameter leaf discs were cut from 50 leaves (care being taken to avoid large leaf veins) and serially washed in 15 changes of sterile distilled water (2 min per washing). The leaf discs were then dried on sterile filter paper and treated as follows: 50 were plated on MA^+, 2 to a plate, and 50 distributed between 10 sterile moist chambers (Keyworth 1951). The agar plates and moist chambers were incubated under fluorescent light at room temperature (21–23°C). Developing fungal colonies were directly identified or transferred to an appropriate medium for identification. Preliminary experiments had shown 15 washings to be adequate for the removal of fungal propagules but very few fungal propagules were removed by the washing method. This was thought to be due to their paucity on the aspen poplar leaf surface rather than to the inefficiency of the washing method.

(b) Surface-sterilized Leaves

Internal fungi were determined by a surface-sterilization process. One-hundred 5-mm diameter leaf discs cut from 50 leaves (care being taken to avoid large leaf veins) were surface sterilized for 30 s in 0.1% (w/v) sterile $AgNO_3$, given two 2-min washes in 0.5% (w/v) NaCl, and five 2-min washes in sterile distilled water. The leaf discs were dried on sterile filter paper and treated as in the plating-out procedure of the washed leaves outlined above. Preliminary experiments using surface-sterilization times of 30, 60, 90, and 120 s had shown them to have equivalent surface-sterilization efficiencies, hence 30 s was chosen for the study.

For the washing (*a*) and surface-sterilization (*b*) experiments, the fungi were analyzed for their frequency of occurrence at each sampling time to determine if there was a successional pattern for the mycoflora of aspen poplar leaves.

A two-way analysis of variance (ANOVA) without replication was applied to some of the data to test for significance of variation among the sampling times and heights, and among the three subplots sampled. The three subplots were then treated as statistical replicates, means of frequency were computed for each fungus, and the Student–Newman–Keuls (SNK) test was applied to test for the differences among the means.

Data having significantly heterogeneous variances (as tested for by Bartlett's and F-max test), hence not conforming to one of the assumptions of the ANOVA and SNK tests, were transformed using common transformations e.g., logarithm of $x + 1$,

LG10 (x + 1.0); square root of $x + \frac{1}{2}$, SQRT (x + 0.5), and retested for homogeneity. The transformation best rendering the sample variances homogeneous and independent of the sample means was used for the analyses.

(c) Leaf-surface Impressions

Leaf-surface impressions were made to complement the foregoing cultural studies with direct observations. Five leaf discs 1.6 cm in diameter were removed from both the upper and lower surfaces of the leaves. The discs were painted with nail varnish which was peeled off when dry and mounted in lactophenol – cotton blue. Each peel was observed under the microscope (×516) and 20 microscope fields were examined by an arbitrary, sequential method. The first field was selected towards the edge of the peel. The next field was selected an arbitrary short distance to one side, and the process continued so the peel was observed in rows of fields. Bissett (1975) has compared this method of selecting fields with a random method and found no significant difference in results when measuring hyphal lengths in agar films. The spore types and mycelium present were recorded and measured with the aid of a drawing tube attached to a microscope and a map measurer. The data had significantly heterogeneous variances (as tested by Bartlett's and F-max test) not to conform to one of the assumptions of the ANOVA and SNK tests. Variances could not be rendered homogeneous and independent of the sample means by the use of several common transformations (e.g., LG10(x + 1.0), SQRT(x + 0.5), sine, cosine, arctangent (ATAN $\sqrt{x}$), and exponential). As an alternative, nonparametric tests were used since they do not assume an underlying normality of the data and their null hypothesis is not concerned with the mean as in the ANOVA but only with the distribution of the variates. A Kruskall–Wallis one-way analysis of variance (K–W ANOVA) and Mann–Whitney U test – Wilcoxon Rank Sum W test were applied to the data to test for differences among the means.

(d) Air Mycoflora Sampling

A comparison between the air mycoflora within the tree canopy and the mycoflora of aspen poplar leaves was made on three occasions during the season. Times for air sampling were chosen to coincide as closely as possible to leaf sampling times (viz. 18 May, 12–13 August, and 7 October) but on days with little or no wind. Sampling was conducted after at least 3 clear, dry days to allow a "dry-air spora" (Gregory 1973) to become established. The air mycoflora was measured at a 2-m height (a standard height for air mycoflora studies) on all occasions and, in addition, at 8 and 12 m on the latter two sampling dates. Sampling was effected using the sticky-slide and gravity Petri dish methods. The inherent weaknesses of these methods as pointed out by Gregory (1973) are appreciated, but they are convenient and economical, and when used together they provide at least a restricted picture of the organisms present in the air.

The sticky-slide method of Lamb and Brown (1970) was used. Five microscope slides coated with a mixture of paraffin wax and white petroleum jelly (2:1, v/v) were presterilized by an ethylene oxide treatment, exposed vertically in the tree canopy for 4 h, and then taken to the laboratory and imprinted onto MA^+ plates. The Petri dish method involved exposing five Petri dishes of MA^+ to the air for 5 min before returning them to the laboratory for incubation. Preliminary experiments revealed that 5 min was adequate to avoid overcrowding of the plate on the one hand and too few colonies on the other. Both the sticky-slide imprinted Petri dishes and the gravity Petri dishes were incubated under fluorescent light at room temperature. Developing fungal colonies were directly identified or transferred to an appropriate medium for identification.

Following Derrick and McLennan (1963), data for the exposed Petri dishes and for the slide-imprinted Petri dishes were expressed in two ways. (1) Percentage of the Petri dishes on which each fungus appeared. Derrick and McLennan noted that in an analysis of colonies only, some important genera could appear of minor significance. (2) Average number of colonies of each fungus per dish.

The data had significantly heterogeneous variances not to conform to one of the assumptions of the ANOVA and SNK tests and variances could not be rendered homogeneous and independent of the sample means by common transformations. The nonparametric K–W ANOVA and Mann–Whitney U test – Wilcoxon Rank Sum W test were applied to the data to test for differences among the means.

Results

The Fungal Succession

The examination of attached aspen poplar leaves revealed the presence of fungi from the opening of buds until leaf fall.

The bud scales appeared to be colonized by a limited range of fungi (Tables 2 and 3). A sterile dark species (No. 2), *Acremonium charticola*, and *Aureobasidium pullulans* were all isolated from washed bud scales. An examination of the bud scale impressions (Figs. 1 and 2) revealed large numbers of *Cladosporium cladosporioides* spores, yeast cells, and many clusters and chains of cells. These clusters and chains of cells were thought to be *Aureobasidium* and were similar to published pictures of *Aureobasidium* on leaf and wood surfaces (Lindsey and Pugh 1976; Stadelmann 1976; Schmidt 1976). There were few germinated spores on the bud scales, those present being *C. cladosporioides*. There was ca. 12.2 cm of fungal mycelium on each square centimetre of bud scale at this time indicating extensive fungal growth on the overwintering bud scales. The lack of fungal isolations from washed scales would suggest that much of this mycelium was nonviable at the time of sampling. Indeed, an examination of some of the bud scale mycelium with the aid of a scanning electron microscope showed much of it to be damaged. There were no fungal isolations from the surface-sterilized bud scales (Tables 4 and 5) suggesting only superficial colonization by fungi.

Few fungi were isolated from the washed leaves enclosed within the bud scales. Those fungi isolated were thought to originate from fungal propagules trapped in the leaf hairs of the few leaves protruding from the buds at the sampling time. Leaf-surface impressions of these enclosed leaves were not possible on a large scale due to the fragile nature and awkward shape (curled) of the material. However those impressions taken revealed no fungal material on the enclosed leaves. Surface sterilization of these leaves revealed no internal leaf colonizers.

TABLE 2. Percentage frequency of occurrence of fungi on aspen poplar leaves throughout the season at 5-m height in the tree canopy as indicated by washed discs plated onto MA+

Fungi isolated	Bud scales	Young leaves	Mature leaves	Senescent leaves	Freshly fallen leaves
Sterile dark species 2*	7.3*a*‡	0.7*a*	0.0*a*	0.0*a*	0.0*a*
Acremonium charticola (Lindau) W. Gams	1.3*a*	14.0*b*	0.0*a*	0.0*a*	0.0*a*
Pink yeasts	0.0*a*	0.7*a*	4.7*a*	0.0*a*	0.0*a*
White yeasts*	0.0*a*	0.7*a*	9.3*b*	6.0*ab*	0.0*a*
Penicillium spp.	0.0*a*	1.3*a*	0.0*a*	1.3*a*	0.0*a*
Cytospora chrysosperma Fr.*	0.0*a*	14.0*b*	0.0*a*	2.7*a*	0.0*a*
Aureobasidium pullulans† (de Bary) Arnauld	4.7*a*	0.7*a*	48.0*b*	42.7*b*	8.0*a*
Cladosporium herbarum (Pers.) Link ex S. F. Gray	0.0*a*	4.0*a*	36.7*b*	26.7*b*	37.3*b*
Cladosporium cladosporioides (Fresen.) de Vries	0.0*a*	4.7*a*	30.0*b*	20.7*bc*	16.0*c*
Sterile dark species 3	0.0*a*	0.0*a*	19.3*b*	4.0*a*	0.0*a*
Botrytis cinerea Pers. ex Pers.	0.0*a*	0.0*a*	6.7*b*	1.3*a*	0.0*a*
Epicoccum purpurascens Enrenb. ex Schlecht	0.0*a*	0.0*a*	0.7*a*	3.3*a*	0.0*a*
Alternaria alternata (Fr.) Keissler	0.0*a*	0.0*a*	0.7*a*	3.3*a*	3.3*a*
Sterile dark species 6	0.0*a*	0.0*a*	0.0*a*	9.3*b*	0.0*a*
Ceuthospora sp.	0.0*a*	0.0*a*	0.0*a*	4.7*b*	0.0*a*
Sterile dark species 1*	0.0*a*	0.7*a*	0.0*a*	28.7*b*	53.3*c*

*Data underwent LG10(x + 1.0) transformation to obtain homogeneous variances.
†Data underwent SQRT(x + 0.5) transformation to obtain homogeneous variances.
‡For each fungus, means sharing the same letter are not significantly different at the 5% level according to SNK test performed on the data.

TABLE 3. Percentage frequency of occurrence of fungi on aspen poplar leaves throughout the season at 5-m height in the tree canopy as indicated by washed discs incubated in moist chambers

Fungi isolated	Bud scales	Young leaves	Mature leaves	Senescent leaves	Freshly fallen leaves
Sterile dark species 2	—†	—	—	—	—
Acremonium charticola	0.0*a*‡	8.7*b*	0.0*a*	0.0*a*	2.0*a*
Pink yeasts	—	—	—	—	—
White yeasts	—	—	—	—	—
Penicillium spp.	0.0*a*	1.3*a*	0.0*a*	0.7*a*	0.7*a*
Cytospora chrysosperma	0.0*a*	4.7*a*	0.0*a*	0.0*a*	0.0*a*
*Aureobasidium pullulans**	0.0*a*	0.0*a*	8.0*bc*	15.3*c*	4.0*b*
*Cladosporium herbarum**	0.0*a*	4.7*b*	30.7*c*	46.7*c*	38.0*c*
Cladosporium cladosporioides	0.0*a*	4.0*a*	26.0*b*	20.7*b*	30.7*b*
Sterile dark species 3	0.0*a*	0.0*a*	1.3*a*	2.0*a*	6.7*b*
Botrytis cinerea	0.0*a*	0.0*a*	1.3*a*	0.0*a*	0.0*a*
Epicoccum purpurascens	0.0*a*	0.0*a*	8.7*b*	2.7*a*	0.0*a*
Alternaria alternata	0.0*a*	0.0*a*	18.7*b*	4.0*c*	1.3*ac*
Sterile dark species 6	—	—	—	—	—
Ceuthospora sp.	—	—	—	—	—
Sterile dark species 1	0.0*a*	0.7*a*	0.7*a*	1.3*a*	12.7*b*
Arthrinium state of *Apiospora montagnei* Sacc.*	0.0*a*	0.7*a*	0.0*a*	0.0*a*	3.3*a*
Beauveria bassiana (Bals.) Vuill.	0.0*a*	3.3*a*	0.0*a*	0.0*a*	0.0*a*
Paecilomyces farinosus (Dickson ex Fr.) Brown & Smith	0.0*a*	0.0*a*	0.7*a*	0.0*a*	0.0*a*
Acremonium sp.	0.0*a*	0.0*a*	0.7*a*	0.0*a*	0.0*a*
Cladosporium macrocarpum Preuss	0.0*a*	0.0*a*	0.0*a*	1.3*a*	0.0*a*
Sterile dark species 7*	0.0*a*	0.0*a*	0.0*a*	6.7*a*	1.3*a*

*Data underwent LG10(x + 1.0) transformation to obtain homogeneous variances.
†A fungus not isolated by this method is indicated by — through the season.
‡For each fungus, means sharing the same letter are not significantly different at the 5% level according to SNK test performed on the data.

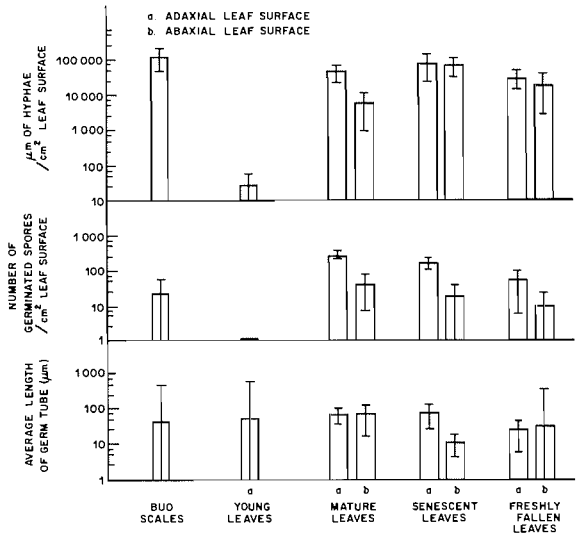

FIG. 1. Number of germinated spores and detail of vegetative structures in leaf-surface impressions of aspen poplar leaves at 5-m height in the tree canopy. The 95% confidence limits are indicated by the lines above and below the bars.

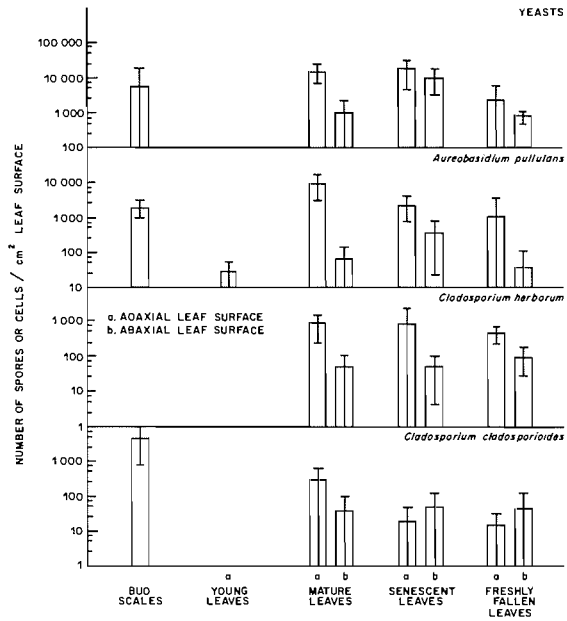

FIG. 2. Numbers of spores and cells recorded in leaf-surface impressions of aspen poplar leaves at 5-m height in the tree canopy. The 95% confidence limits are indicated by the lines above and below the bars.

The young newly expanded leaves had very little fungal mycelium present on them and few germinated spores (Fig. 1). *Acremonium charticola* and *Cytospora chrysosperma* were the most frequently isolated fungi from washed leaf discs at this time (Tables 2 and 3). Pink and white yeasts, *Aureobasidium pullulans*, *Cladosporium herbarum*, *C. cladosporioides*, *Beauveria bassiana*, the *Arthrinium* state of *Apiospora montagnei*, and *Penicillium* spp. were all isolated in low frequencies by the cultural methods. The spores of these fungi were not found to any great extent (if at all) in the leaf-surface impressions (Fig. 2). Surface-sterilization experiments failed to show any internal leaf colonizers (Tables 4 and 5). It appeared that the newly expanded aspen poplar leaves were sparsely colonized by fungi.

Air mycoflora studies revealed the presence of viable fungal propagules in the air (Tables 6 and 7) so it would appear that the young leaves were acting as a newly presented sterile (with respect to fungi) landing stage for fungal propagules. Almost all the fungi isolated from washed leaf discs and hence presumably actively growing and sporulating, were also isolated from the air mycoflora.

The mature aspen poplar leaves were extensively colonized by phylloplane fungi. White yeasts, *Aureobasidium pullulans*, *Cladosporium herbarum*, *C. cladosporioides*, *Epicoccum purpurascens*, *Alternaria alternata*, *Botrytis cinerea*, and a sterile dark species (No. 3) were all isolated from washed leaf discs in significantly ($P = 0.05$) greater frequencies in August than on the newly expanded leaves sampled in June (Tables 2 and 3).

The leaf-surface impressions taken in August revealed extensive hyphal development on the mature leaves (Fig. 1). There was ca. 4.5 and 0.6 cm of fungal mycelium per square centimetre (cm/cm^2) of adaxial and abaxial leaf surface, respectively. The numbers of spores and cells of phylloplane fungi showed a significant ($P = 0.05$) increase with increasing leaf age (Figs. 1 and 2). Generally, spore and cell numbers were greater on the adaxial than the abaxial surface of the leaves.

The surface-sterilization experiments showed no significant internal colonization of the mature leaves by the commonly accepted phylloplane fungi, with the exception of *C. cladosporioides* (Tables 4 and 5). However, a sterile dark species (No. 1) was isolated in high frequency.

In the August air mycoflora samples (Tables 6 and 7) the presence was noted of most of the common phylloplane fungi (viz. *Cladosporium* spp., *Aureobasidium*, *Penicillium* spp., *Epicoccum*, *Alternaria*, and *Botrytis*); however, *Beauveria bassiana*, *Cytospora chrysosperma*, *Acremonium charticola*, and *Verticillium* spp. were absent, and these fungi were also apparently absent from the phylloplane at the August (mature leaf) sample time.

The leaf-washing method indicated that with a few exceptions, the fungi on the mature leaves

TABLE 4. Percentage frequency of occurrence of fungi within aspen poplar leaves throughout the season at 5-m height in the tree canopy as indicated by surface sterilized discs plated onto MA+

Fungi isolated	Bud scales	Young leaves	Mature leaves	Senescent leaves	Freshly fallen leaves
Sterile dark species 1*	0.0*a*†	0.0*a*	29.3*b*	24.7*b*	69.3*c*
Cladosporium herbarum	0.0*a*	0.0*a*	1.3*a*	16.0*b*	0.7*a*
Cladosporium cladosporioides	0.0*a*	0.0*a*	3.3*b*	6.0*c*	0.7*a*
Epicoccum purpurascens	0.0*a*	0.0*a*	0.7*a*	1.3*a*	0.0*a*
Aureobasidium pullulans	0.0*a*	0.0*a*	0.0*a*	3.3*a*	0.7*a*
Pink yeasts*	0.0*a*	0.0*a*	0.0*a*	0.0*a*	4.0*a*
Penicillium spp.	0.0*a*	0.7*a*	0.0*a*	0.0*a*	0.0*a*
Cladosporium macrocarpum	0.0*a*	0.0*a*	0.0*a*	2.0*a*	0.0*a*
Alternaria alternata	0.0*a*	0.0*a*	0.0*a*	1.3*a*	0.0*a*
Botrytis cinerea	0.0*a*	0.0*a*	0.0*a*	0.7*a*	0.0*a*
Sterile dark species 6	0.0*a*	0.0*a*	0.0*a*	13.3*b*	0.0*a*
Sterile dark species 14*	0.0*a*	0.0*a*	0.0*a*	8.7*a*	0.0*a*
Sterile dark species 8	0.0*a*	0.0*a*	0.0*a*	4.7*b*	0.0*a*
Sterile dark species 15	0.0*a*	0.0*a*	0.0*a*	4.0*a*	0.0*a*
Sterile dark species 17	0.0*a*	0.0*a*	0.0*a*	4.0*b*	0.0*a*
Sterile dark species 7	0.0*a*	0.0*a*	0.0*a*	2.7*b*	0.0*a*
Sterile dark species 3	0.0*a*	0.0*a*	0.0*a*	1.3*a*	0.0*a*

*Data underwent LG10(x + 1.0) transformation to obtain homogeneous variances.
†For each fungus, means sharing the same letter are not significantly different at the 5% level according to SNK test performed on the data.

TABLE 5. Percentage frequency of occurrence of fungi within aspen poplar leaves throughout the season at 5-m height in the tree canopy as indicated by surface sterilized discs incubated in moist chambers

Fungi isolated	Bud scales	Young leaves	Mature leaves	Senescent leaves	Freshly fallen leaves
Sterile dark species 1	0.0*a*†	0.0*a*	28.0*b*	10.7*a*	58.0*c*
*Cladosporium herbarum**	0.0*a*	0.0*a*	0.0*a*	19.3*b*	0.7*a*
Cladosporium cladosporioides	0.0*a*	0.0*a*	0.7*a*	10.0*b*	0.0*a*
Epicoccum purpurascens	0.0*a*	0.0*a*	0.0*a*	2.7*a*	0.0*a*
Aureobasidium pullulans	0.0*a*	0.0*a*	0.0*a*	1.3*a*	0.0*a*
Pink yeasts	—‡	—	—	—	—
Penicillium spp.	0.0*a*	0.0*a*	1.3*a*	0.0*a*	0.0*a*
Cladosporium macrocarpum	—	—	—	—	—
Alternaria alternata	0.0*a*	0.0*a*	0.0*a*	2.0*a*	2.7*a*
Botrytis cinerea	—	—	—	—	—
Sterile dark species 6	0.0*a*	0.0*a*	0.0*a*	5.3*a*	0.0*a*
Sterile dark species 14	—	—	—	—	—
Sterile dark species 8	—	—	—	—	—
Sterile dark species 15	—	—	—	—	—
Sterile dark species 17	—	—	—	—	—
Sterile dark species 7	0.0*a*	0.0*a*	0.0*a*	0.7*a*	0.0*a*
Sterile dark species 3*	0.0*a*	0.0*a*	0.0*a*	12.7*b*	0.0*a*
Acremonium charticola	0.0*a*	0.0*a*	0.0*a*	3.3*a*	2.0*a*
Sterile dark species 9	0.0*a*	0.0*a*	1.3*a*	2.7*a*	0.0*a*
Sterile dark species 39*	0.0*a*	0.0*a*	6.7*a*	0.0*a*	0.0*a*
Arthrinium state of *Apiospora montagnei*	0.0*a*	0.0*a*	0.0*a*	1.3*a*	0.0*a*
Sterile dark species 44	0.0*a*	0.0*a*	0.0*a*	3.3*a*	0.0*a*

*Data underwent LG10(x + 1.0) transformation to obtain homogeneous variances.
†For each fungus, means sharing the same letter are not significantly different at the 5% level according to SNK test performed on the data.
‡A fungus not isolated by this method is indicated by — throughout the season.

continued colonizing the senescing aspen poplar leaves (Tables 2 and 3). Sterile dark species 3, *Botrytis cinerea*, *Epicoccum purpurascens*, and *Alternaria alternata* all showed significant (P = 0.05) declines in frequency of occurrence on the washed senescent leaf discs. A *Ceuthospora* species, sterile dark species 6, and sterile dark species 1 all increased (P = 0.05) in frequency with leaf senescence.

Leaf-surface impressions of the senescent leaves

TABLE 6. Occurrence of fungi on exposed Petri dishes at three heights in the tree canopy

Fungi isolated	18 May at sampling height of 2 m		12–13 August at sampling height of 2 m		12–13 August at sampling height of 8 m		12–13 August at sampling height of 12 m		7 October at sampling height of 2 m		7 October at sampling height of 8 m		7 October at sampling height of 12 m	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Pink yeasts					13	(0.13)			13	(0.13)			13	(0.20)
White yeasts	60	(0.80)	20	(0.27)	13	(0.20)	15	(0.15)	60	(0.80)	87	(1.27)	80	(1.53)
(Other) yeasts	7	(0.07)							7	(0.07)			7	(0.07)
Cladosporium herbarum	27	(0.60)	67	(0.93)	53	(0.87)	40	(0.73)	60	(1.20)	73	(1.53)	67	(1.60)
Cladosporium cladosporioides	13	(0.33)	53	(1.13)	27	(0.27)	32	(0.48)	67	(1.40)	73	(1.80)	93	(4.00)
Aureobasidium pullulans			13	(0.13)	7	(0.07)	7	(0.07)	7	(0.20)	7	(0.07)	33	(0.40)
Penicillia–Aspergilli			40	(2.53)	33	(0.47)	23	(0.77)	47	(3.67)	27	(0.53)	33	(0.87)
Sterile hyaline spp.	20	(0.20)	80	(1.07)	27	(0.27)	52	(0.65)	33	(0.60)	27	(0.47)	4	(0.47)
Sterile dark spp.	33	(0.53)	27	(0.40)	20	(0.33)	38	(0.75)	60	(1.07)	47	(0.73)	4	(0.53)
Epicoccum purpurascens					7	(0.07)								
Beauveria bassiana	7	(0.07)											13	(0.13)
Acremonium charticola – Verticillium spp.	13	(0.27)									7	(0.07)	20	(0.20)
Cladosporium macrocarpum									20	(0.33)	13	(0.27)	60	(2.93)
Botrytis cinerea											7	(0.07)	7	(0.07)
Cytospora chrysosperma											13	(0.20)		
Trichoderma harzianum Rifai											7	(0.07)		
Oidiodendron sp.													7	(0.07)
Alternaria alternata – Ulocladium atrum Preuss													7	(0.13)

NOTE: K–W ANOVA revealed no significant differences with respect to time or height for the fungal species examined. (Exception: *Cladosporium cladosporioides* (*a*) — $P = 0.038$ with respect to time.) (*a*) Percentage of exposed dishes on which fungus occurred. (*b*) Average number of colonies per dish are in parentheses.

TABLE 7. Occurrence of fungi on exposed sticky slides at three heights in the tree canopy

Fungi isolated	18 May at sampling height of 2 m		12–13 August at sampling height of 2 m		12–13 August at sampling height of 8 m		12–13 August at sampling height of 12 m		7 October at sampling height of 2 m		7 October at sampling height of 8 m		7 October at sampling height of 12 m	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Pink yeasts	7	(0.20)	37	(0.37)	15	(0.15)	7	(0.07)					7	(0.07)
White yeasts	75	(1.64)	37	(0.87)	43	(0.63)	40	(0.87)	73	(1.40)	47	(0.47)	27	(0.33)
(Other) yeasts					8	(0.08)	7	(0.07)						
Cladosporia	57	(1.71)	100	(5.43)	100	(5.70)	93	(4.27)	80	(2.87)	93	(1.80)	80	(2.07)
Aureobasidium pullulans	38	(0.58)	7	(0.07)	28	(0.35)	7	(0.07)	27	(0.40)	13	(0.13)	13	(0.13)
Penicillia–Aspergilli	31	(0.38)	38	(0.62)	40	(1.23)	13	(0.13)	7	(0.07)	13	(0.13)	13	(0.20)
Sterile hyaline spp.	42	(0.91)			15	(0.22)	20	(0.20)	27	(0.33)	40	(0.40)	20	(0.20)
Sterile dark spp.	55	(0.69)	22	(0.22)	15	(0.22)	20	(0.33)	47	(0.60)	27	(0.20)	27	(0.27)
Epicoccum purpurascens					7	(0.07)								
Beauveria bassiana	18	(0.18)											7	(0.07)
Acremonium charticola – Verticillium spp.	62	(2.20)												
Botrytis cinerea			35	(0.40)	20	(0.40)	40	(0.40)						
Cytospora chrysosperma														
Alternaria alternata – Ulocladium atrum	7	(0.07)	7	(0.07)			7	(0.07)						
Chrysosporium sp.					8	(0.08)								

NOTE: K–W ANOVA revealed no significant differences with respect to time or height for the fungal species examined. (Exceptions: pink yeasts (*a*) — $P = 0.046$ with respect to time; *Botrytis cinerea* (*a*) and (*b*) — $P = 0.022$ and $P = 0.021$, respectively, with respect to time; sterile hyaline species (*b*) — $P = 0.042$ with respect to time.) (*a*) Percentage of exposed slides on which fungus occurred. (*b*) Average number of colonies per slide is in parentheses.

(Figs. 1 and 2) showed that there was ca. 7.6 cm /cm^2 of fungal mycelium on the adaxial surface, that is, no significant change from the amount on mature leaves. However, there was an increase in the amount of fungal mycelium on the abaxial leaf surface, from ca. 0.6 cm/cm^2 on mature leaves to ca. 6.9 cm/cm^2 on senescent leaves. With some exceptions, spore and cell numbers on both leaf surfaces remained essentially unchanged from the levels found on the mature leaves (Figs. 1 and 2).

As the leaves senesced, a greater internal colonization by phylloplane fungi occurred (Tables 4 and 5). The sterile dark species 1 still occurred in high frequency and *C. herbarum*, *C. cladosporioides*, and several sterile dark species were isolated in significantly ($P = 0.05$) greater numbers. In fact many fungi, including several other common phylloplane fungi (viz. *Aureobasidium*, *Epicoccum*, and *Alternaria*) were isolated in low frequency from the surface-sterilized senescent leaf discs (Tables 4 and 5).

Sampling of the air mycoflora was undertaken at a time intermediate between the times of sampling of the senescent leaves and the freshly fallen leaves for phylloplane studies, and once again, all the common phylloplane fungi were isolated (Tables 6 and 7).

The freshly fallen aspen poplar leaves appeared to be less extensively colonized by the commonly accepted phylloplane fungi than when they were on the tree (Tables 2 and 3). The *Cladosporium* spp. were isolated from washed leaf discs in the same high frequencies as they were from the mature and senescent leaves. Sterile dark species 1 was the most frequently recovered fungus from washed leaf discs. The white yeasts, *Aureobasidium*, *Ceuthospora* sp., and sterile dark species 6 all showed significant ($P = 0.05$) reductions in frequency of isolation from the leaf discs at this time.

The leaf-surface impressions of the freshly fallen leaves showed several interesting trends (Figs. 1 and 2). The amount of fungal mycelium on the leaves had declined from the level at leaf senescence. There was a nonsignificant decline in the amount on the adaxial surface (from ca. 7.6 to ca. 2.9 cm/cm^2) but there was a significant ($P = 0.05$) drop on the abaxial surface with leaf fall (from ca. 6.9 to ca. 2.0 cm/cm^2). Some of the mycelium observed in the leaf-surface impressions appeared to have undergone autolysis or to have been grazed by insects. This was also confirmed by scanning electron microscopy observations. Numbers of *Cladosporium* spores on the leaves remained unchanged with leaf fall. Numbers of yeast and *Aureobasidium* cells on both leaf surfaces declined with leaf fall, confirming the observations on their behaviour from the washed leaf discs. In general, however, spore numbers on both leaf surfaces remained unchanged as the senescent leaves fell from the tree. Large numbers of an unknown spore type (thought to be a rust urediniospore) were seen in the leaf-surface impressions at this time.

The surface-sterilization experiments showed that the freshly fallen aspen poplar leaves were colonized internally by sterile dark sp. 1 and by little else (Tables 4 and 5).

The Effect of Leaf Height in the Tree Canopy on the Phylloplane Fungi

The cultural isolation of fungi from washed leaf discs revealed that height in the tree canopy had some influence on the phylloplane fungi of aspen poplar leaves (Table 8). Several fungi (*Cytospora chrysosperma*, *Ceuthospora* sp., and sterile dark species 1) showed significant ($P = 0.05$) changes in frequency of isolation with time, and three (pink yeasts and sterile dark species 3 and 6) showed time × height interactions. *Botrytis cinerea* revealed significant ($P = 0.05$) changes with both time and height, and *Aureobasidium pullulans* had a significant ($P = 0.05$) change in frequency of isolation with height in the canopy. Both *Botrytis* and *Aureobasidium* showed a progressive decline in frequency with sampling height. The majority of fungi, however, revealed no significant changes in frequency of isolation between the two sample times and three heights examined.

The leaf-surface impressions revealed that on the adaxial surface of mature leaves there was a decline in fungal mycelial lengths with increasing leaf height in the tree canopy (Fig. 3). The abaxial surface had no such changes with height in the canopy. The mycelial lengths on the abaxial surface were significantly ($P = 0.05$) lower than those on the adaxial surface except at the 12-m sampling height. The differences in lengths of fungal mycelium between leaf surfaces and with differing leaf height in the tree canopy had essentially disappeared on the senescent leaves.

Yeast and *Aureobasidium* cell numbers, and numbers of *Cladosporium herbarum* and *C. cladosporioides* spores all behaved in a similar way with respect to time and height (Fig. 4). On the adaxial surfaces of both mature and senescent leaves, numbers were greatest at the 5-m height. Values at 8 and 12 m were similar, but less than those at 5 m. There were no significant changes with height in numbers on the abaxial surface at

TABLE 8. Percentage frequency of occurrence of fungi on aspen poplar leaves at three heights in the tree canopy on two occasions in the season as indicated by washed discs plated onto MA+

Fungi isolated	Mature leaves at height in canopy of			Senescent leaves at height in canopy of			Variance ratios from two-way analysis of variance on data		
	5 m	8 m	12 m	5 m	8 m	12 m	Time × height	Time	Height
Sterile dark species 2	0.0	0.0	0.0	0.0	0.0	0.0	NS†	NS	NS
Acremonium charticola	0.0	0.7	0.0	0.0	0.0	0.0	NS	NS	NS
Pink yeasts	4.7	0.0	0.0	0.0	0.0	0.7	*	NS	NS
White yeasts	9.3	4.7	2.7	6.0	4.7	0.7	NS	NS	NS
Penicillium spp.	0.0	0.0	0.7	1.3	0.0	0.0	NS	NS	NS
Cytospora chrysosperma	0.0	0.0	0.0	2.7	2.0	1.3	NS	*	NS
Aureobasidium pullulans‡	48.0	21.3	4.0	42.7	32.7	4.0	NS	NS	***§
Cladosporium herbarum	36.7	34.7	50.7	26.7	50.0	46.0	NS	NS	NS
Cladosporium cladosporioides	30.0	33.3	22.7	20.7	18.7	22.7	NS	NS	NS
Sterile dark species 3	19.3	8.7	16.0	4.0	4.7	3.3	*	**	NS
Botrytis cinerea	6.7	2.0	0.7	1.3	0.0	0.0	NS	*	*
Epicoccum purpurascens	0.7	2.7	2.7	3.3	4.0	1.3	NS	NS	NS
Alternaria alternata	0.7	2.0	1.3	3.3	0.0	0.7	NS	NS	NS
Sterile dark species 6	0.0	0.0	0.0	9.3	0.0	0.0	**	***	**
Ceuthospora sp.	0.0	0.0	0.0	4.7	3.3	0.0	NS	*	NS
Sterile dark species 1‡	0.0	0.0	0.7	28.7	25.3	32.0	NS	**	NS

NOTE: Levels of significance: $*P < 0.05$; $**P < 0.001$; $***P < 0.01$.
†NS, not significant.
‡Data underwent $LG10(x + 1.0)$ transformation to obtain homogeneous variances.
§The two-way ANOVA analysis revealed significant change with height. Percentage frequencies of occurrence at the two times were combined and the data analyzed by one-way ANOVA and SNK tests. No transformation applied would obtain homogeneous variances with this combined data. As the variances approached homogeneity (as tested by Bartlett's and F-max test), the values separated as 5 > 8 > 12 m.

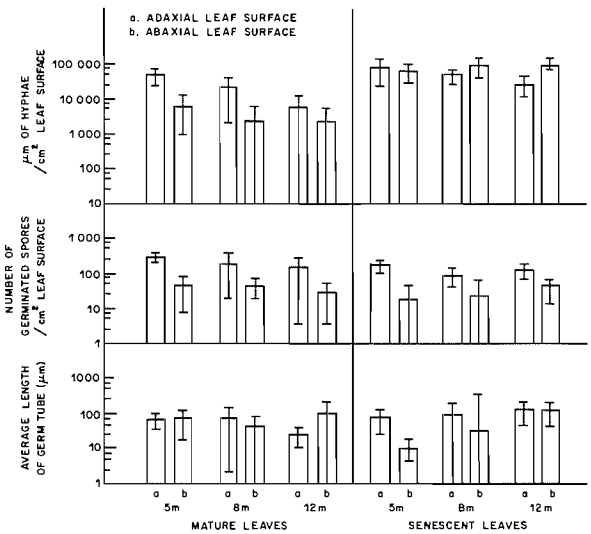

FIG. 3. Number of germinated spores and detail of vegetative structures in leaf-surface impressions of aspen poplar leaves at three heights in the tree canopy on two occasions in the season. The 95% confidence limits are indicated by the lines above and below the bars.

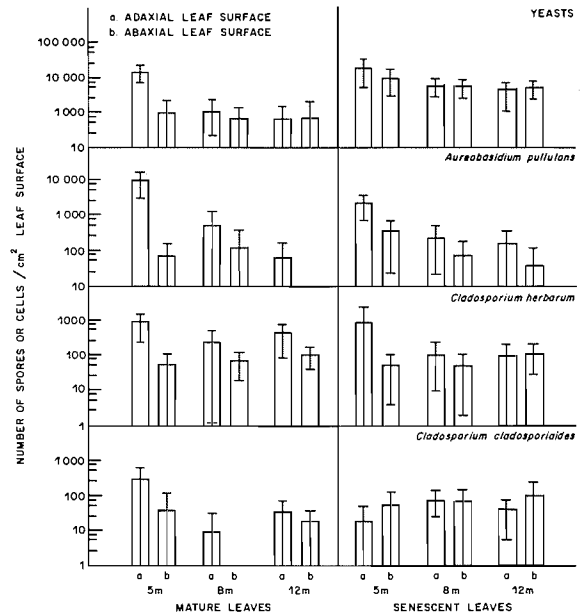

FIG. 4. Numbers of spores and cells recorded in leaf-surface impressions of aspen poplar leaves at three heights in the tree canopy on two occasions in the season. The 95% confidence limits are indicated by the lines above and below the bars.

either sampling time. At the 5-m height in the tree canopy, numbers were greater ($P = 0.05$) on the adaxial leaf surface, but at 8 and 12 m, numbers were similar on both leaf surfaces. In general, the numbers of other spores on the mature and senescent leaves were similar at all three sampling heights and on both sides of the leaf.

An examination of the surface-sterilization data (Table 9) revealed little influence of leaf height in the tree canopy on the internal leaf mycoflora. Several fungi (*Aureobasidium pullulans* and sterile dark species 8, 15, and 7) showed significant ($P = 0.05$) changes with time. Three fungi (sterile dark spp. 1, 6, and 17) showed time × height interac-

TABLE 9. Percentage frequency of occurrence of fungi within aspen poplar leaves at three heights in the tree canopy on two occasions in the season as indicated by surface sterilized discs plated onto MA+

Fungi isolated	Mature leaves at height in canopy of			Senescent leaves at height in canopy of			Variance ratios from two-way analysis of variance on data		
	5 m	8 m	12 m	5 m	8 m	12 m	Time × height	Time	Height
Sterile dark species 1	29.3	10.7	21.3	24.7	39.3	52.0	*	**	NS
Cladosporium herbarum†	1.3	5.3	4.0	16.0	28.0	32.7	NS	***	**
Cladosporium cladosporioides	3.3	4.0	1.3	6.0	8.0	2.7	NS	*	*
Epicoccum purpurascens	0.7	0.0	0.7	1.3	0.7	0.0	NS	NS	NS
Aureobasidium pullulans	0.0	0.0	0.0	3.3	4.0	0.0	NS	*	NS
Pink yeasts	0.0	0.0	0.0	0.0	0.7	0.0	NS	NS	NS
Penicillium spp.	0.0	2.0	0.0	0.0	0.0	0.0	NS	NS	NS
Cladosporium macrocarpum	0.0	0.0	0.7	2.0	0.0	0.7	NS	NS	NS
Alternaria alternata	0.0	0.0	0.0	1.3	0.0	0.0	NS	NS	NS
Botrytis cinerea	0.0	0.0	0.0	0.7	0.7	0.7	NS	NS	NS
Sterile dark species 6†	0.0	0.0	0.0	13.3	6.7	0.7	**	***	**
Sterile dark species 14†	0.0	0.0	0.0	8.7	0.7	0.0	NS	NS	NS
Sterile dark species 8	0.0	0.0	0.0	4.7	6.0	6.0	NS	**	NS
Sterile dark species 15	0.0	0.0	0.0	4.0	0.7	0.0	NS	*	NS
Sterile dark species 17	0.0	0.0	0.0	4.0	1.3	0.0	**	**	**
Sterile dark species 7	0.0	0.0	0.0	2.7	2.7	3.3	NS	**	NS
Sterile dark species 3	0.0	0.0	0.0	1.3	0.7	0.7	NS	NS	NS
Sterile dark species 39	0.0	0.7	0.0	0.0	0.0	0.0	NS	NS	NS

NOTE: Levels of significance: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
†Data underwent $LG10(x + 1.0)$ transformation to obtain homogeneous variances.
‡NS, not significant.

tions. *Cladosporium herbarum* showed significant ($P = 0.05$) changes with both time and height. The isolation frequencies at 8 and 12 m were higher than the value at 5 m. *Cladosporium cladosporioides* also showed significant ($P = 0.05$) changes with both time and height. However, the frequency of isolation at 12 m was lower than the values at 5 and 8 m. Little or no change in the air mycoflora with height in the tree canopy was found in the present investigation (Tables 6 and 7).

Discussion

A superficial colonization of aspen poplar bud scales by fungi was observed in the present investigation. Warren (1976) also observed that colonization by bacteria and fungi (white and pink yeasts, *Aureobasidium pullulans*, and *Cladosporium* spp.) was confined to the outer bud scales of *Populus trichocarpa*. Unfortunately, Warren's washing and homogenization method did not distinguish between fungi on the bud scale and fungi within the bud scale tissue.

Few of the successional studies of fungi on leaves have included measurements of hyphal lengths, and spore and cell numbers throughout a season. Those that have, however, (Dickinson 1967; Stadelmann 1976; Bernstein and Carroll 1977; McBride and Hayes 1977) have found a general increase in hyphal development with increasing leaf age. Differences in hyphal development between upper and lower leaf surfaces have been noted (Stadelmann 1976; Bernstein and Carroll 1977; McBride and Hayes 1977).

The young, newly expanded aspen poplar leaves were sparsely colonized by fungi. In effect, they were a mycologically clean landing stage for the air mycoflora to alight upon. The fungal propagules showed a tendency to adhere more frequently on the adaxial leaf surface. As a consequence, the hyphal development on the mature leaves was greater on the adaxial leaf surface. The numbers of fungal propagules was also greater on the adaxial surface at this time, possibly due to sporulation of the actively growing fungi and to the abovementioned tendency for fungal propagules to adhere to that surface.

Hudson's (1968) general scheme for colonization of nonlignicolous substrates by fungi notes that primary saprophytic colonists are ascomycetes and fungi imperfecti of which the more common ones are *Cladosporium herbarum*, *Alternaria alternata*, *Epicoccum purpurascens*, *Aureobasidium pullulans*, and *Botrytis cinerea*. These are considered to arrive at the host surface as airborne spores and only become vegetatively active with the onset of host senescence. However, the present investigation and others (Dickinson 1965; Ruscoe 1971; Pugh and Buckley 1971; Pugh and Mulder 1971; Godfrey 1974; Lindsey and Pugh 1976; Dickinson 1976) have indicated the onset of vegetative activity of these phylloplane fungi prior to leaf senescence.

Accompanying leaf senescence there was an in-

crease in the amount of fungal mycelium, but only on the abaxial leaf surface. It seemed that some factor(s) were limiting the mycelial development on the adaxial surface. One possible factor limiting growth may have been freely available carbon. This may also have halted further development of yeast and *Aureobasidium* cells on the upper surfaces of senescing leaves. This concept will be further discussed in another paper (H. G. Wildman and D. Parkinson, submitted for publication). It should be emphasized that limiting carbon may have been only one of several factors operating here. Spore numbers on the senescing leaves were greater on the adaxial leaf surface, as had been found for mature leaves. There was a gradual increase in internal colonization of the leaves with senescence indicating a slow penetration of the leaf interior with time. Greater invasion of senescent leaf tissue by phylloplane fungi has also been noted in several previous studies (Kerling 1958; Ruscoe 1971; Pugh and Buckley 1971; Lindsey and Pugh 1976).

With leaf fall there was a marked change in the mycoflora of the aspen poplar leaves. There was a slight decline in the amount of mycelium on the adaxial leaf surface and a substantial decrease in the length of mycelium on the abaxial surface. Possible reason(s) for the decline in mycelial lengths being primarily restricted to the abaxial surface may have been: (*i*) preferential autolysis on this side of the leaf just prior to leaf fall, and (or) (*ii*) preferential grazing by insects on the abaxial surface mycelium. The decline in yeast and *Aureobasidium* cell numbers with leaf abscission may have been due to the dryness of the fallen leaves. Bashi and Fokkema (1977) have shown yeast cell numbers on leaves to be sensitive to relative humidity. Ruscoe (1971) used direct observation techniques to show *Aureobasidium* to have colonized the phylloplane of young *Nothofagus* leaves and to have formed vigorously growing colonies whose development increased with increasing leaf age and then declined at leaf fall. After leaf fall, the colonization of the aspen poplar leaf interior was almost solely by one fungal species.

As the air mycoflora was sampled on only three occasions, the patterns of behaviour of the fungi do not become readily apparent. In fact, the species complement of the air mycoflora seemed remarkably constant with time. More frequent sampling would be needed to detect any subtle changes in it. The study has shown that there was a source of viable fungal propagules in the atmosphere throughout the season. The bulk of the air mycoflora was comprised of genera which were also isolated from the aspen poplar phylloplane. Similar observations have been made by other workers (Last and Deighton 1965; Collins and Hayes 1976).

The height of the leaves in the tree canopy was shown to influence the amount of fungal mycelium on, and the mycoflora of, the aspen poplar leaves, with certain species (the yeasts, *Aureobasidium pullulans*, *Cladosporium* spp., and *Botrytis cinerea*) showing changes in their frequency with sampling height.

The progressive decline of total hyphal lengths and of yeast and *Aureobasidium* cell numbers on the adaxial surface of mature leaves with increasing leaf height in the tree canopy may have been due to limiting carbon. On the abaxial surface at this time, the mycelium, yeast cells, and *Aureobasidium* cells were still developing and were perhaps not carbon limited, hence they showed no significant changes with leaf height in the canopy (this is further discussed by Wildman and Parkinson (submitted for publication)). The decline in numbers of *Cladosporium* spp. spores on the adaxial leaf surface with increasing leaf height in the tree canopy at this stage may have been the result of two processes: (*i*) although the number of viable *Cladosporium* spp. propagules appeared constant with height in the tree canopy (Tables 6 and 7) there may have been a preferential adhesion on the lowermost leaves in the canopy, and (or) (*ii*) accompanying the progressive decline in total mycelium with increasing leaf height in the tree canopy there was a progressive decline in actively growing and sporulating *Cladosporium* mycelium.

At leaf senescence mycelial lengths on the leaves were constant with increasing leaf height in the tree canopy, but the numbers of yeast and *Aureobasidium* cells were lower on the adaxial leaf surface at 8 and 12 m than at 5 m. Several factors appeared to be influencing the development of the phylloplane mycoflora at this stage (this is further discussed in Wildman and Parkinson (submitted for publication)).

Lamb and Brown (1970) found the density of fungal propagules in the air within the crown of *Eucalyptus stellulata* to be constant with respect to height. Similarly, the height above ground level had little influence on the mycoflora on leaves of the eucalypt. Little or no change in the air mycoflora with height in the tree canopy was found in the present investigation (Tables 6 and 7). As pointed out earlier, however, more intensive sampling may be needed to observe any subtle changes in the air mycoflora at different heights in the canopy.

Bernstein and Carroll (1977) noted that microbial

populations might be expected to respond to variations in temperature, humidity and light, to the relative availability of dissolved substances in canopy wash, and to differing grazing pressures. They mentioned that marked differences in all of these parameters had been found from one location in the tree canopy to the next. Further studies of these parameters and their influence on the phylloplane microorganisms are needed.

The phylloplane is a complex habitat and the pitfalls of oversimplification must be avoided in attempts to understand it.

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BASHI, E., and N. J. FOKKEMA. 1977. Environmental factors limiting growth of *Sporobolomyces toseus*, an antagonist of *Cochliobolus sativus*, on wheat leaves. Trans. Br. Mycol. Soc. **68**: 17–25.

BERNSTEIN, M. E., and G. E. CARROLL. 1977. Microbial populations on Douglas-fir needle surfaces. Microbial Ecol. **4**: 41–52.

BISSETT, J. D. 1975. Ecology of fungi occurring in soils along an alpine ridge. Ph.D. thesis, University of Calgary, Calgary.

COLLINS, M. A., and A. J. HAYES. 1976. Seasonal incidence of microbes on the surface of first year needles of Norway spruce. Trans. Br. Mycol. Soc. **66**: 457–461.

DICKINSON, C. H. 1965. The mycoflora associated with *Halimione portulacoides*. III. Fungi on green and moribund leaves. Trans. Br. Mycol. Soc. **48**: 603–610.

——— 1967. Fungal colonization of *Pisum* leaves. Can. J. Bot. **45**: 915–927.

——— 1976. Fungi on the aerial surfaces of higher plants. *In* Microbiology of aerial plant surfaces. *Edited by* C. H. Dickinson and T. F. Preece. Academic Press, London. pp. 293–324.

DICKINSON, C. H., and J. O'DONNELL. 1977. Behaviour of phylloplane fungi on *Phaseolus* leaves. Trans. Br. Mycol. Soc. **68**: 193–199.

DICKINSON, C. H., and T. F. PREECE. 1976. Microbiology of aerial plant surfaces. Academic Press, London.

DICKINSON, C. H., and G. J. F. PUGH. 1974. Biology of plant litter decomposition. Vols. I and II. Academic Press, London.

DICKINSON, C. H., and B. WALLACE. 1976. Effects of late applications of foliar fungicides on activity on microorganisms on winter wheat flag leaves. Trans. Br. Mycol. Soc. **76**: 103–112.

DERRICK, E., and E. I. MCLENNAN. 1963. Fungus spores found in the air in Melbourne (Victoria), Australia. Acta Allergol. **18**: 26–43.

GREGORY, P. H. 1973. The microbiology of the atmosphere. 2nd ed. Leonard Hill, London.

GODFREY, B. E. S. 1974. Phylloplane mycoflora of bracken, *Pteridium aquilinum*. Trans. Br. Mycol. Soc. **62**: 305–311.

HUDSON, H. J. 1968. The ecology of plant remains above the soil. New Phytol. **67**: 837–874.

KERLING, L. C. P. 1958. De microflora op het blad van *Beta vulgaris* L. Tijdschr. Plantenziekten, **64**: 402–410.

——— 1964. Fungi in the phyllosphere of leaves of rye and strawberry. Meded. Landbouwhogesch. Opzoekingsstn. Staat Gent, **29**: 885–895.

KEYWORTH, W. G. 1951. A Petri dish moist chamber. Trans. Br. Mycol. Soc. **34**: 291–292.

LAMB, R. J., and J. F. BROWN. 1970. Nonparasitic microflora on leaf surfaces of *Paspalum dilatatum Salix babylonica*, and *Eucalyptus stellulata*. Trans. Br. Mycol. Soc. **55**: 383–390.

LAST, F. T., and F. C. DEIGHTON. 1965. The nonparasitic microflora on the surface of living leaves. Trans. Br. Mycol. Soc. **58**: 329–331.

LAST, F. T., and R. C. WARREN. 1972. Nonparasitic microbes colonizing green leaves; their form and functions. Endeavour, **31**: 143–150.

LINDSEY, B. I., and G. J. F. PUGH. 1976. Succession of microfungi on attached leaves of *Hippophae rhamnoides*. Trans. Br. Mycol. Soc. **67**: 61–67.

LOUSIER, J. D., and D. PARKINSON. 1976. Litter decomposition in a cool temperate deciduous forest. Can. J. Bot. **54**: 419–436.

——— 1978. Chemical element dynamics in decomposing leaf litter. Can. J. Bot. **56**: 2795–2812.

MCBRIDE, R. P., and A. J. HAYES. 1977. Phylloplane of European larch. Trans. Br. Mycol. Soc. **69**: 39–46.

MITCHELL, M. J. 1974. Ecology of oribatid mites (*Acari: Cryptostigmata*) in an aspen woodland soil. Ph.D. thesis, University of Calgary, Calgary.

PREECE, T. F., and C. H. DICKINSON. 1971. Ecology of leaf surface microorganisms. Academic Press, London.

PUGH, G. J. F., and N. G. BUCKLEY. 1971. The leaf surface as a substrate for the colonization by fungi. *In* Ecology of leaf surface microorganisms. *Edited by* T. F. Preece and C. H. Dickinson. Academic Press, London. pp. 431–445.

PUGH, G. J. F., and J. L. MULDER. 1971. Mycoflora associated with *Typha latifolia*. Trans. Br. Mycol. Soc. **57**: 273–282.

RUSCOE, Q. W. 1971. Mycoflora of living and dead leaves of *Nothofagus truncata*. Trans. Br. Mycol. Soc. **56**: 463–474.

SCHMIDT, E. L. 1976. *Aureobasidium pullulans* on wood shingles. For. Prod. J. **26**: 34–37.

SHARMA, K. R., and K. G. MUKERJI. 1973. Microbial colonization of aerial parts of plants—a review. Acta Phytopathol. Acad. Sci. Hung. **8**: 425–461.

RUINEN, J. 1961. The phyllosphere. I. An ecologically neglected milieu. Plant Soil, **15**: 81–109.

STADELMANN, F. 1976. Die Apfel—und Birnen—Phyllosphären—Mikroflora und ihre Beeinflussung durch biotische und abiotische Faktoren, insbesondere durch *Venturia inaequalis* (Cke.) Wint. und *Venturia pirina* (Bref.) Aderh. Ph.D. thesis, Universität Basel, Basel.

TUITE, J. 1969. Plant pathological methods—fungi and bacteria. Burgess Publishing Co, Minneapolis, MN.

VISSER, S., and D. PARKINSON. 1975. Fungal succession on aspen poplar leaf litter. Can. J. Bot. **53**: 1640–1651.

WARREN, R. C. 1976. The occurrence of microbes among buds of deciduous trees. Eur. J. For. Pathol. **6**: 38–45.